Characterizing the pBad promoter with Arabinose

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Because we are using pBad as our promoter to start the cascade of responses in our system, we needed to test the leakiness of the promoter. To do this, we put pBad on a 1C3 plasmid with Red Fluorescent Protein (RFP).

The promoter, pBad, turns on in the presence of arabinose and in doing so, allows RFP to be transcribed. Therefore, once arabinose is present, our cells will fluoresce red.

The amount that our cells fluoresce depends on the amount of arabinose. To further characterize this observation, we grew up overnights with varying concentrations of arabinose and measured their fluorescence.

Protocol:

For the characterization of fluorescence of pBad + RFP stimulated by arabinose, use a 0% - 0.2% arabinose concentration.

Note: Increments of 0.04% concentration is recommended.

1. Make six 5mL overnights (with antibiotic) with varying concentrations of arabinose: 0, 0.04, 0.08, 0.12, 0.16, and 0.2 percent.

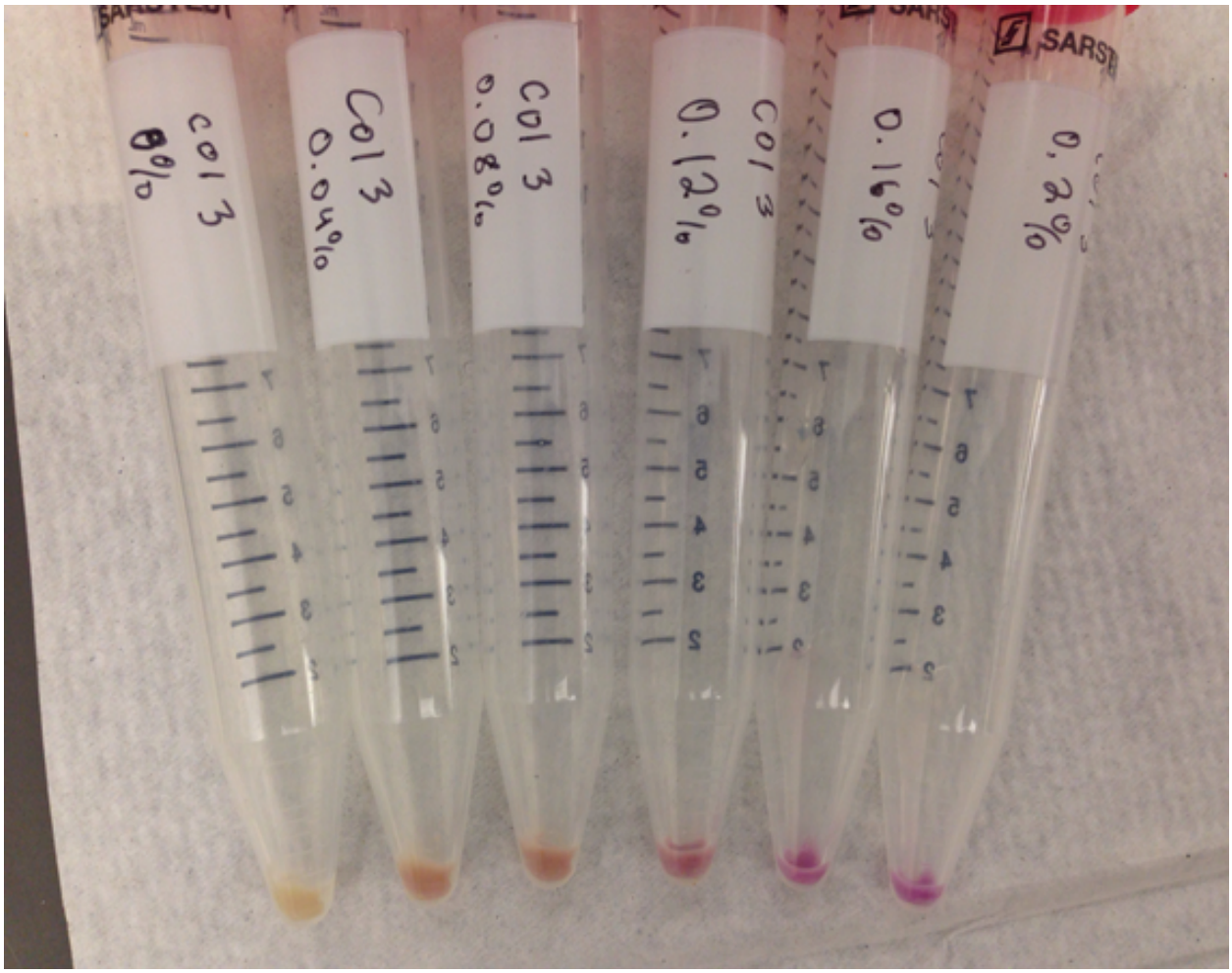
2. Add cells containing pBad + RFP plasmid.
Note: to start with a consistent amount of cells in each tube, make one overnight of the cells. Use this culture of cells to inoculate the six characterization tubes (using equal volumes for all six). Keep in mind to take very small amounts since the overnight will contain a high concentration of cells.

3. Let cells grow for at least 12 hours.

4. Spin cells down (3 minutes at 8,000 x g).

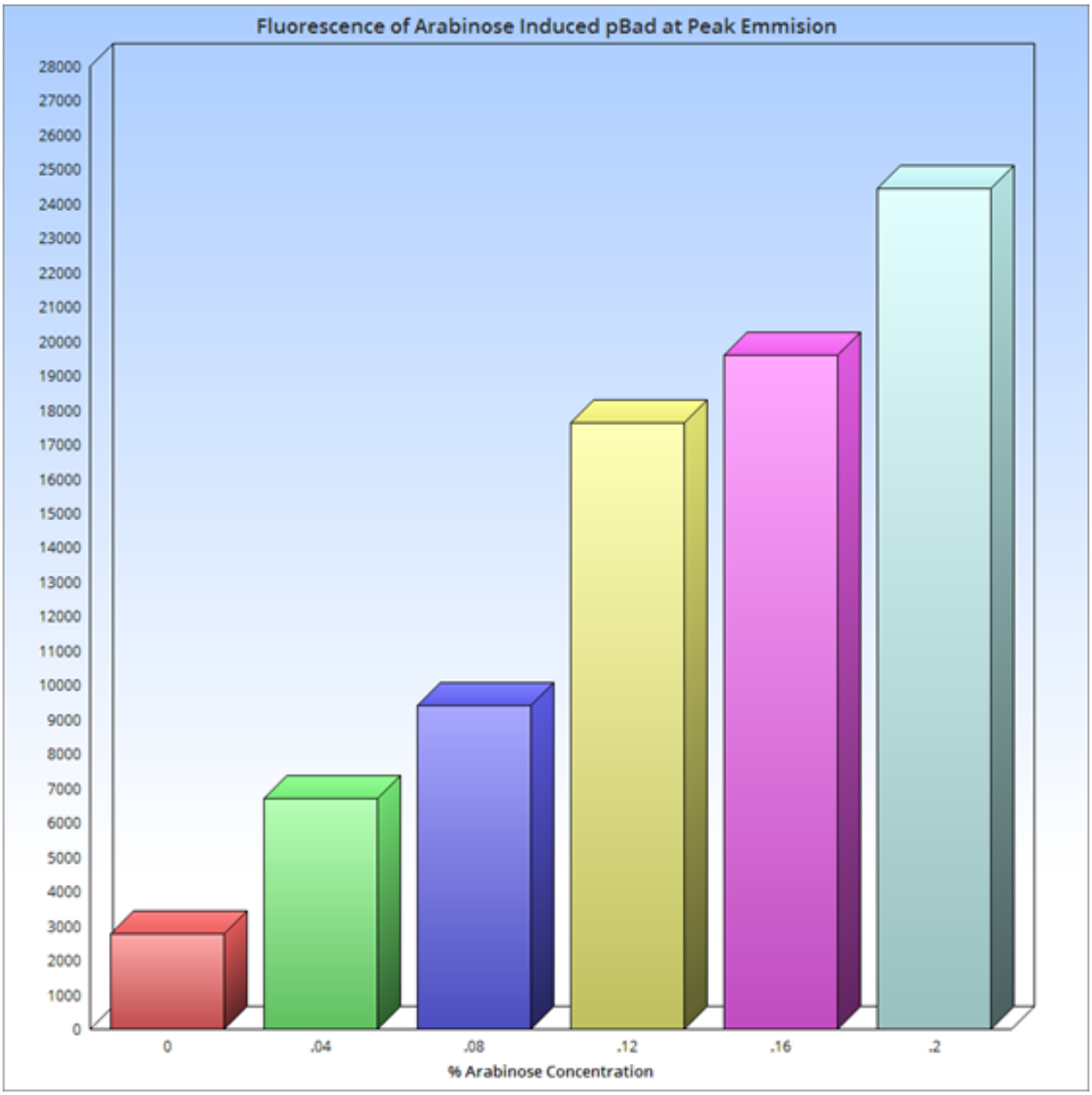
5. Decant.

6. Lay tubes next to each other in order of arabinose concentration and compare brightness of fluorescence by eye. Take photo to document.



Varied concentrations of arabinose from 0% to 0.2% by 0.04% increments (7/14/15)

Then we used flow cytometry to more accurately characterize the fluorescence:



As illustrated in the flow cytometry characterization, we do see some cells fluorescing even when no arabinose is present, therefore the pBad promoter is leaky.

Although the promoter is leaky, it is sufficient for our proof of concept at this stage of our project. If the leakiness needed to be addressed, the promoter could be transferred to a lower copy plasmid or one could use a weaker pBAD promoter such as BBa_K206001, thereby reducing the effects of leakiness.